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Genotype-Phenotype Correlations in Cornelia de Lange syndrome: Behavioral Characteristics and Changes with Age

by

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Running Head: Genotype-Phenotype Correlations in CdLS

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ABSTRACT

Cornelia de Lange syndrome (CdLS) is a multisystem genetic disorder associated with unusual facial features, limb abnormalities, a wide range of health conditions and intellectual disability. Mutations in five genes that encode (*SMC1A, SMC3, RAD21*) or regulate (*NIPBL*, *HDAC8*) the cohesin complex have been identified in up to 70% of individuals. Genetic cause remains unknown for a proportion of individuals. There is substantial heterogeneity in all aspects of CdLS but very little is known about what predicts phenotypic heterogeneity. In this study we evaluated genotype-phenotype associations in thirty-four individuals with CdLS. Participants with *NIPBL* mutations had significantly lower self help skills and were less likely to have verbal skills relative to those who were negative for the *NIPBL* mutation. No significant differences were identified between the groups in relation to repetitive behavior, mood, interest and pleasure, challenging behavior, activity, impulsivity and characteristics of autism spectrum disorder whilst controlling differences in self help skills. Significant correlations indicating lower mood, interest and pleasure and increased insistence on sameness with older age were identified for those who were *NIPBL* mutation positive. The findings suggest similarities in the behavioral phenotype between those with and without the *NIPBL* mutation once differences in self help skills are controlled for. However, there may be subtle differences in the developmental trajectory of these behaviors according to genetic mutation status in CdLS.

Keywords: behavioral phenotype, Cornelia de Lange syndrome, CdLS, genotype-phenotype correlation, NIPBL.

INTRODUCTION

Cornelia de Lange syndrome (CdLS) is a rare multisystem genetic disorder that affects approximately one child in every 40,000-100,000 [O'Brien & Yule, 1995]. The syndrome is associated with unusual facial features, limb malformations [Selicorni et al., 2007] and a wide range of health conditions [Hall et al., 2008]. Associated intellectual disability (ID) is typically within the severe to profound range, although a proportion of individuals may have moderate or mild ID [Sloneem et al., 2009]. Behavioral characteristics include social avoidance, repetitive and self-injurious behaviors and hyperactivity [Berney et al., 1999; Hyman et al., 2002; Moss et al., 2009; Oliver et al., 2008]. Autism spectrum disorder (ASD) characteristics are common, and may be extensive enough to warrant diagnosis of an ASD in 51% -67% of individuals [Oliver et al., 2008; Basile et al., 2007; Bhuiyan et al., 2007; Moss et al., 2008; Oliver et al., 2011; Strivastava et al., 2014; Nakanishi et al., 2012]. More recently, signs of premature ageing and changes in behavior, mood and cognition with age have been described [Oliver et al., 2011; Kline et al., 2007; Nelson et al., 2014; Reid, 2010]. There is substantial heterogeneity in all aspects of CdLS but very little is known about what predicts phenotypic heterogeneity. Understanding this is crucial to the early identification of those individuals with CdLS who are at greater risk of developing cognitive, behavioral and emotional difficulties and to guide appropriate, targeted clinical intervention and management.

The most common known genetic cause of CdLS is a mutation in *NIPBL*, which accounts for up to 80% of cases [Krantz et al., 2004; Tonkin et al., 2004; Huisman et al., 2013]. Mosaicism for *NIPBL* mutations is identified in 23% of individuals [Huisman et al., 2013]. A number of other less common causal mutations have also been identified. Mutations in *SMC1a* and *SMC3* have been found to account for CdLS in a further 5% of affected individuals [Deardorff et al., 2007; Musio et al., 2006], and more recently, mutations in

HDAC8 and *RAD21* have been identified in a small number of cases [Deardorff et al., 2012a; Deardorff et al., 2012b]. All of these genes are thought to encode proteins related to cohesin complex function.

Studies that have reported genotype-phenotype correlations in CdLS have primarily described variability in clinical and diagnostic characteristics within and between mutation variants. The general consensus is that individuals with *NIPBL* mutations are likely to present with more severe clinical features and to have more impaired cognitive function than those with other causal mutations and those for whom mutations have not been identified, although this is not always the case [Gillis et al., 2004; Mannini et al., 2013 Nakanishi et al., 2012]. Those with *SMC* mutations are generally described as presenting with a 'milder' CdLS phenotype, moderate cognitive impairment and fewer structural abnormalities than those with NIPBL mutations [Deardorff et al., 2007; Gil-Rodríguez et al., 2015; Pié et al., 2010]. Individuals with *RAD21* mutations demonstrate a somewhat subtle clinical presentation with a very mild cognitive impairment [Deardorff et al., 2012b], while those with *HDAC8* mutations are considered to be more similar to those with *NIPBL* mutations but with fewer limb abnormalities and other possible clinical features that may distinguish them from other individuals with CdLS [Mannini et al., 2013; Kline et al., 2014].

Studies evaluating genotype-phenotype correlations with regard to behavioral characteristics are more limited. Gil-Rodríguez and colleagues [2015] described fewer behavioral problems in those with *SMC3* mutations, although no standardized assessments of behavior were employed and there was no comparison between individuals with different CdLS mutation variants. Nakanishi et al. [2012] described a trend for higher scores on the Autism Diagnostic Interview-Revised [ADI-R; Rutter, LeCouteur and Lord 2003] in individuals with *NIPBL* mutations compared to those without an identified mutation, although this difference was not statistically significant.

In the current study we aimed to evaluate genotype-phenotype associations in relation to a broad range of behavioral features known to be characteristic of CdLS including: challenging behavior, ASD characteristics, mood and hyperactivity. Specifically, we compared individuals with a confirmed *NIPBL* mutation to those for whom the *NIPBL* mutation was not identified. A secondary aim was to explore the effect of mutation status on potential changes with age that have been reported in the literature.

MATERIALS AND METHODS

Procedure

The study was approved by the West Midlands Coventry and Warwickshire Research Ethics Committee. Participants were identified from a pre-existing database of 252 individuals with CdLS who had taken part in questionnaire surveys as part of a larger research project evaluating behavioral characteristics in neurodevelopmental disorders [Arron et al., 2011; Moss et al., 2009; Nelson et al., 2013; Oliver et al., 2011]. These participants had originally been recruited via the CdLS Foundation (UK and Ireland) or via a pre-existing participant database held at the University of Birmingham, UK. The total number of individuals with CdLS approached in the original studies was 376. Of these, 116 participants (response rate of 30.85%) responded and took part. A further 108 individuals responded to follow up calls for participation in these (and other related) studies between 2006 and 2012. A total of 126 individuals had provided their consent for the researchers at the University of Birmingham to contact other relevant professionals in order to confirm diagnostic status, including ascertaining the results of mutation analyses, where these had been carried out.

The results from mutation analyses were sought from two clinics in the UK; the MRC Human Genetics Unit, University of Edinburgh and the Northern Regional Genetics Service,

Newcastle. These are the only two clinics in the UK where genetic testing for CdLS is routinely conducted. A total of 24 participants had previously been tested at one or other of these clinics (Edinburgh n= 12, Newcastle n=10; DNA sequencing failed in two further participants) and agreed that data could be shared. Of the remaining participants (n=102), 83 were contacted by the research team by phone and by letter and were invited to participate in a genetic screening study at the Human Genetics Unit, Edinburgh (nineteen participants did not have up to date contact details and could not be reached for this purpose). Mutation analyses were performed for a further twelve participants through this screening study and these data shared. This resulted in a total sample of 34 individuals for whom both questionnaire data regarding behavioral characteristics and data from mutation analyses were available. All participants had a confirmed clinical diagnosis of CdLS from a clinical geneticist. The recruitment strategy is summarized in Figure 1.

+++Insert Fig 1 about here+++

Participants

Participant characteristics and behavioral responses are summarized in Table I. *NIPBL* mutations were confirmed in seventeen individuals (50.00 % of total sample), one individual had an *HDAC8* mutation (2.94%) and three had a *SMC1a* mutation (8.82%). Five of the participants for whom a *NIPBL* mutation was not detected had not received further screening for other CdLS mutations because these were not routinely carried out within that particular service. Eight participants were negative for all known CdLS mutations. These participants were evaluated using the Average Face Analysis described by Ansari et al. [2014]. Based on this, four of the participants were classified as 'unlikely *NIPBL*' and one was classified as '*NIPBL*-like'. There was insufficient information available for the Average Face Analysis for three individuals.

+++Insert Table I about here+++

Participants who had only been tested for *NIPBL* mutations and were found to be *NIPBL* negative (n=5), those participants who were found to be negative for all mutations but had insufficient information for the Average Face Analysis (n=3) and participants under the age of four years (n=4) were excluded from the following analyses.

Measures

Demographic information including date of birth, gender, mobility, verbal ability (i.e. able to communicate more than 30 signs/words) and diagnostic status (by whom and when) was collected using a brief background questionnaire.

The Wessex Scale [Kushlick et al., 1973] provides a proxy measure of adaptive behavior skills. The measure evaluates the physical and social abilities of individuals on subscales including self-help skills, continence, mobility, speech and literacy. The measure has good inter-rater reliability with children and adults, at both the item and subscale level [Kushlick et al., 1973; Palmer & Jenkins, 1982].

The Activity Questionnaire [TAQ; Burbidge et al., 2010] evaluates hyperactivity and impulsivity in individuals with intellectual disability and is suitable for use with both non-verbal and verbal individuals. The questionnaire consists of 18 items across three subscales: impulsivity, over-activity and impulsive speech. For the purposes of this study, items requiring speech were excluded from the analysis in order to account for group differences in verbal skills. Robust internal consistency and reliability has been established by the authors.

The Repetitive Behaviour Questionnaire [RBQ; Moss et al., 2009] identifies specific types of repetitive behavior in both children and adults with intellectual disabilities. The

questionnaire is made up of nineteen operationally defined and observable behaviors across five subscales: restricted preferences, repetitive speech, insistence on sameness, stereotyped behavior, and compulsive behavior. A five point Likert rating scale is used to record responses which range from 'never' to 'more than once a day'. For the purposes of this study, items requiring speech were excluded from the analysis in order to account for group differences in verbal skills. Other studies have shown the questionnaire to have good reliability and validity [Moss et al., 2009].

The Challenging Behaviour Questionnaire [CBQ; Hyman et al., 2002] is a brief measure designed to assess the presence or absence of challenging behaviors over the past month including physical and verbal aggression, self-injury and destruction of property. Good inter-rater reliability has been established [Hyman et al., 2002]. The CBQ is derived from the Challenging Behaviour Interview which is also reported to have good reliability and validity [Oliver et al., 2003].

The Mood, Interest and Pleasure Questionnaire Short Version [MIPQ-S; Ross and Oliver, 2003; Arron et al., 2011] evaluates two constructs associated with depression in adults and children with intellectual disabilities. Informants are required to rate 12 items based on retrospective observations over a two week period. The questionnaire shows good internal consistency and reliability [Ross and Oliver, 2003].

The Social Communication Questionnaire [SCQ; Rutter et al., 2003] is a screening tool designed to measure communication and social skills in participants suspected of having ASD. The questionnaire comprises three subscales: communication, social interaction and repetitive and stereotyped behaviors. A proportional communication score [as per Moss et al., 2013 and Warner et al., 2014] was employed for nonverbal individuals in order to ensure comparability across verbal and non-verbal participants. Higher scores are indicative of more

significant ASD characteristics. A cut off score of 15 is suggested by the authors to be indicative of ASD, while a score of 22 indicates the presence of Autism. These cut off scores do not reflect clinical diagnosis. The SCQ has been shown to have good concurrent validity with the Autism Diagnostic Observation Schedule and Autism Diagnostic Interview [Berument et al., 1999; Howlin and Karpf, 2004].

Data analysis

To evaluate genotype-phenotype correlations, between group comparisons were conducted contrasting the clinical characteristics (self-help skills, mobility, hearing, vision and speech) and scores on the behavioral assessments of those participants who had a confirmed mutation in *NIPBL* (*NIPBL*-positive) and those for whom a mutation in *NIPBL* had not been identified (*NIPBL*-negative; includes participants who were positive for *SMC1a* and *HDAC8* mutations). Chi squared tests were conducted for categorical data and independent samples t-tests (or nonparametric equivalent when data were not normally distributed) or analysis of covariance were conducted for continuous variables (or variables which could be treated as continuous), with self-help skills as a covariate. Given previous reports within the literature of changes with age in CdLS, Pearson correlations were performed between chronological age and behavioral variables within each mutation status group (*NIPBL*-positive, *NIPBL*-negative).

RESULTS

Clinical characteristics

Participant characteristics and results from between group analyses are described in Table II. The *NIPBL*-positive group had significantly lower self-help skills than the *NIPBL*-negative group. There were no significant group differences with regard to gender ratio, vision or

mobility between the *NIPBL*-positive and *NIPBL*-negative groups. The *NIPBL*-negative group were significantly more likely to be reported as 'verbal' and group differences in relation to hearing problems approached significance.

+++Insert Table II about here+++

Behavioral characteristics

Table III describes the scores on each of the behavioral questionnaires completed and the results of the between group analyses using self-help skills as a covariate where appropriate. There were no significant group differences in relation to any of these behavioral measures.

+++Insert Table III about here+++

Changes with age

Table IV shows the results from the Pearson correlation analyses between chronological age and scores on behavioral measures in each of the mutation groups. Significant negative correlations between age and interest and pleasure scores on the MIPQ-S (indicating lower interest and pleasure with older age) was identified in the *NIPBL*-positive group only. A significant positive correlation between age and insistence on sameness scores on the RBQ (indicating increased frequency of insistence on sameness with older age) was also identified in the *NIPBL*-positive group.

+++Insert Table IV about here+++

DISCUSSION

In the current study we aimed to describe genotype-phenotype correlations in CdLS, with a specific focus on behavioral characteristics, using standardized behavioral measures. This study utilized pre-existing databases in two specialist genetics centers (the only two centers within the UK that screen for CdLS) and an extensive behavioral database at the University of Birmingham. In total, 34 participants for whom both behavioral and genetic data were available and able to be shared were identified.

Individuals with a confirmed mutation in *NIPBL* (1 participant was classified as *NIPBL*-like based on Average Face Analysis [Ansari et al., 2014]) had significantly lower self-help scores and were more likely to be reported as 'verbal' than those who were *NIPBL* mutation negative. This is consistent with previous reports of greater severity of cognitive impairment in individuals with *NIPBL* [Nakanishi et al., 2012; Gillis et al., 2004; Mannini et al., 2013]. No other differences in clinical characteristics including vision, hearing and mobility were identified.

There were no significant differences between the *NIPBL*-positive and *NIPBL*-negative groups in relation to mood, activity, impulsivity, repetitive behavior, challenging behavior and ASD characteristics when controlling for group differences in self-help skills. Few studies have specifically reported on the behavioral differences observed between individuals with *NIPBL* mutations and those without, using standardized measures of behavior. The current study findings indicate that once differences in self-help skills are accounted for, distinctions between the mutation groups are less prominent.

Scores on the Mood, Interest and Pleasure questionnaire were significantly, negatively correlated with chronological age (indicating lower scores for older participants) in the *NIPBL*-positive group. Insistence on sameness was also significantly correlated with age in

this group (indicating higher rates of insistence on sameness in older participants). Interestingly, these associations were not identified in those who did not have the *NIPBL* mutation. Previous studies have described significant changes in mood and insistence on sameness with age in CdLS [Oliver et al., 2011; Nelson et al., 2014], alongside a number of other behavioral and physical changes [Kline et al., 2007]. Given that the distribution of ages varies between the groups, these findings should be interpreted with caution. However, this exploratory analysis suggests that there may be a degree of specificity for these changes with genetic variation. Variability in the nature and degree to which changes with age manifest across different genetic variations of CdLS has not previously been described but has prominent clinical implications. Identifying those most at risk for changes with age in CdLS would enable early detection, intervention and management for these individuals and ultimately enable improved support for these individuals and their families. Furthermore, the suggestion that the nature of these changes with age may be different in those with different genetic mutations may be important for understanding the etiology of this change and the relevance of genetic mechanism in this pathway. These findings should be evaluated further in a larger study sample in order to confirm the pattern of variability.

The study findings should be considered in the context of a number of limitations. Interpretation of the findings is somewhat limited by the small sample size. However, analyses identified significant mutation group differences and associations despite the small sample size, suggesting that statistical power was sufficient. Previous studies have demonstrated heterogeneity *within* the group of individuals identified as having *NIPBL* mutations, with missense mutations resulting in a milder presentation than deletion, nonsense and splicing mutations [Bhuiyan et al., 2006; Gillis et al., 2004; Mannini et al., 2013; Pié et al., 2010]. The nature of *NIPBL* mutations in the current study sample are outlined in Table 1. However, the sample was not sufficiently large enough to enable group comparisons across

these subtypes. It is likely that the small sample size results from the data collection strategy employed. A retrospective approach was employed in order to 'pool' resources among specialist UK based centers working with individuals with CdLS and their families. This was considered to be the most efficient approach to data collection because it utilized existing data and consequently reduced the burden on families (avoiding repeat DNA analysis and repeating behavioral surveys). Surprisingly, it proved more difficult than expected to combine existing data sets across different research and clinical groups (largely as a result of ethical restrictions imposed on the databases at each research/clinical site), resulting in a relatively small sample size (relative to the size of existing databases and the number of participants who had previously provided consent for information to be shared across different research/clinical groups). Given the rarity of the syndrome and to avoid participant fatigue, researchers and clinicians should collectively consider ways in which this approach might be maximized more effectively in the future. A centralized, national database might be one way in which this could be achieved.

The use of informant based measures, which may be subject to bias, is a limitation of the study, particularly when informants are likely to be aware of the behavioral characteristics associated with the syndrome. Further direct assessments of behavior are required in order to confirm the pattern of similarity and difference between genetic subgroups of CdLS.

In summary, the findings from this study confirm previously identified differences in overall level of ability between those individuals with *NIPBL* mutations and those without. However, there may be subtle differences in the developmental trajectory of behaviors, according to genetic mutation status in CdLS. In particular, individuals with *NIPBL* mutations might be at greater risk for experiencing a decline in interest and pleasure and an increase in insistence on sameness with age. These findings require replication in a larger study sample.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare

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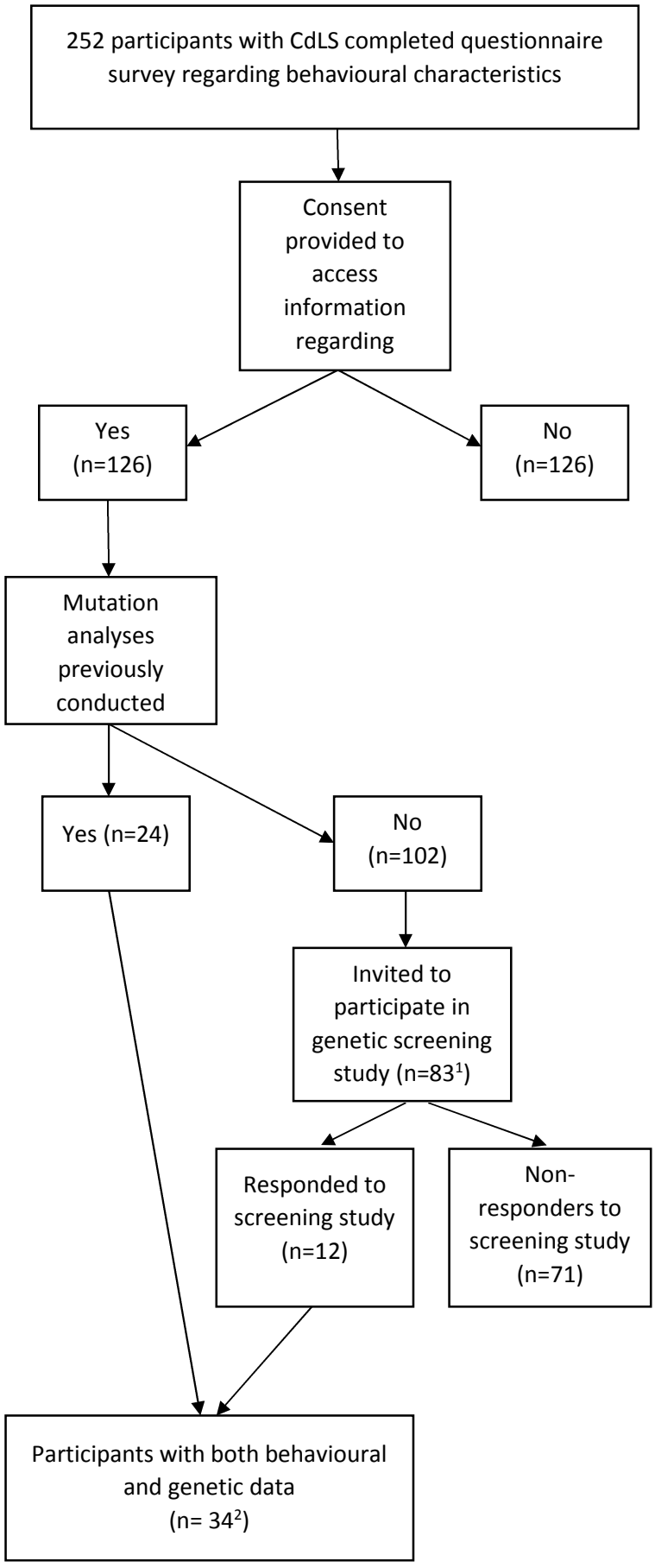

[1] Contact information was not available for 19 participants who had consented to access diagnostic information
[2]DNA sequencing unsuccessful in two participants.

Fig 1: Participant recruitment strategy

Table I: Individual participant characteristics and total scores on behavioral questionnaires

Participant	Age (years)	Gene	Mutation type (as given)	Molecular results	Gender	Average Face Analysis	self-help [3]	SCQ[4]	MIPQ[5]	TAQ[6]	CBQ[7]	RBQ[8]
1	3.00	*NIPBL*	SNV	c.458+3A>C p.?	male	na	4	*	24	42	self-injury aggression property destruction	20
2	3.47	*NIPBL*	SNV (splice site mutation)	c.5808+1G>A p.?	female	na	3	*	33	7	self-injury stereotypies	8
3	4.02	*NIPBL*	mosaic mutation	c.[=/7373_7374del] p.[=/(Ser2458Cysfs*4)]	male	na	4	19.71	38	38	self-injury property destruction stereotypies	19
4	5.41	*NIPBL*	SNV (missense)	c.6069T>G (p.His2023Gln)	male	na	7	10	43	32	self-injury aggression property destruction stereotypies	12
5	5.42	*NIPBL*	SNV (nonsense)	c.1534C>T (p.Gln512X)	female	na	4	11.86	43	12	aggression property destruction	8
6	5.46	*NIPBL*	SNV (splice site mutation)	c.3575-2A>T	female	na	7	14.81	*	38	self-injury aggression property destruction	*
7	7.39	*NIPBL*	SNV (missense)	c.3574G>A (p.Glu1192Lys)	female	na	3	24.79	38	51	self-injury aggression property destruction stereotypies	12
8	8.02	*NIPBL*	SNV (nonsense)	c.6880C>T (p.Gln2294*)	female	na	3	22	33.82	31	property destruction stereotypies	9
9	8.85	*NIPBL*	deletion	c.7653_7655delACA (p.Gln2551del)	female	na	4	24	42	21		4
10	9.82	*NIPBL*	deletion	c.6653_6655del (p.Asn2218del)	male	na	6	29	32	57	self-injury aggression property destruction stereotypies	46
11	10.81	*NIPBL*	deletion	c.157delC (p.Leu53Phefs*25)	male	na	3	23	35	32.5	self-injury aggression stereotypies	13
12	11.58	*NIPBL*	SNV (nonsense)	c.2350C>T p.(Gln784*)	female	na	3	*	27	9	self-injury	4
13	15.63	*NIPBL*	SNV (missense)	c.5464G>A	male	na	7	8	37	16	self-injury	34

14	19.77	*NIPBL*	SNV (missense)	(p.Asp1822Asn) c.6647A>G (p.Tyr2216Cys)	male	na	6	30	29	38	self-injury aggression property destruction stereotypies	27
15	23.00	*NIPBL*	SNV (missense)	c.5464G>C (p.Asp1822His)	female	na	9	7	36	1		18
16	3.00	*NIPBL*	deletion	chr5:36,935,055-37,022,102 x1	male	na	3	*	24	37	self-injury aggression property destruction stereotypies	14
17	24.23	*NIPBL*	SNV (nonsense)	c.3590C>G (p.Ser1197*)	male	na	4	27	30	29	self-injury property destruction stereotypies	12
18	7.48	*HDAC8*	SNV (missense)	c.562G>A (p.Ala188Thr)	female	na	8	16	38	22	self-injury	11
19	9.75	*SMC1A*	mosaic mutation	c.[=/1585_1587del3]; [0] p.[=/(Lys529del)]	male	na	5	21	37	41	self-injury aggression property destruction stereotypies	12
20	17.45	*SMC1A*	SNV (missense)	c.2368C>T (p.Arg790Trp)	female	na	8	16	30	6		3
21	2.33	*SMC1A*	*	*	female	na	4	*	38	27.5	stereotypies	16
22	<1 yr	NMD[1]	na	na	female	na	3	*	29	16	stereotypies	8
23	5.00	NMD[1]	na	na	female	na	7	13	40.36	59	self-injury aggression property destruction	23
24	8.45	NMD[1]	na	na	female	na	3	14	38	57	self-injury aggression	7
25	16.00	NMD[1]	na	na	female	na	3	13.86	38	22	stereotypies	6
26	18.75	NMD[1]	na	na	female	na	7	26	29	24	self-injury aggression property destruction stereotypies	28
27	2.37	NMD[2]	na	na	male	*	5	*	35	52	self-injury aggression property destruction stereotypies	19
28	14.58	NMD[2]	na	na	male	*	9	0	23	45		11
29	18.08	NMD[2]	na	na	male	Unlikely	9	*	40	4	property destruction	10

						NIPBL					stereotypies		
30	18.66	NMD[2]	na	na	female	Unlikely	9	14	35	9		8	
						NIPBL							
31	25.39	NMD[2]	na	na	female	Unlikely	6	23	27.27	43	self-injury	31	
						NIPBL					aggression property destruction stereotypies		
32	30.70	NMD[2]	na	na	female	*NIPBL*-like	4	32	31	45	self-injury stereotypies	31	
33	40.70	NMD[2]	na	na	female	*	4	24	29	27		23	
34	45.45	NMD[2]	na	na	female	Unlikely	9	14	46.91		self-injury	10	
						NIPBL							

NMD= no mutation detected; [1]Tested for NIPBL only; [2]Negative for all known CdLS mutations; [3]Based on Wessex Scale: maximum score = 9; [4]Total Social Communication Questionnaire score (missing items prorated see measures section below): maximum score = 40, cut off for ASD = 15, cut off for Autism =22; [5] Total Mood, Interest and Pleasure Questionnaire score: maximum score=48; [6] Total Activity Questionnaire score excluding impulsive speech: maximum score = 60; [7]Challenging Behaviour Questionnaire; [8]Repetitive Behaviour Questionnaire excluding speech items: maximum score= 60; *information not available

Table II: Participant characteristics

			***NIPBL*-positive (n=15)**	***NIPBL*-negative (n=7)**	**t/χ^2**	**df**	***p***
Age		Mean (SD)	12.67 (8.17)	20.32 (12.58)	-1.72	20	.10
Self help score			4.93 (1.91)	7.71 (1.60)	-3.34	20	.003
Gender	male	N (%)	7 (46.7)	2 (28.6)	*	*	.65
Speech	verbal/partly		6 (40.0)	7 (100.0)	*	*	.02
Mobility	mobile		8 (53.3)	5 (71.4)	*	*	.65
Vision	normal		12 (85.7)	7 (100.0)	*	*	.53
Hearing	normal		8 (53.3)	7 (100.0)	*	*	.05

* Fishers exact

NIPBL-positive: participants with a confirmed *NIPBL* mutation (n=14) or classified by Average Face Analysis as *NIPBL*-like (n=1)

NIPBL-negative: participants who did not have a *NIPBL* mutation (includes those with *SMC1A* (n=3), *HDAC8* (n=1) mutations and those negative for all known CdLS mutations (n=3)

Table III: Group comparisons on measures of behavior

		NIPBL positive (n=15)	NIPBL negative (n=7)	F/U/χ^2	df	*p*
Social Communication Questionnaire						
Social interaction	Mean (SD)	7.50 (4.93)	7.67 (3.01)	1.76	1,20	.20
Communication		9.24 (4.35)	6.88 (2.23)	.96	1,20	.34
Repetitive behavior		4.21 (2.42)	3.00 (1.55)	.58	1,20	.46
Total score		22.89 (10.42)	17.33 (3.78)	.30	1,20	.59
ASD cut off**	N (%)	9 (64.30)	4 (66.7)	*	*	1.00
Autism cut off**		9 (64.30)	1 (16.7)	*	*	.14
Challenging Behaviour Questionnaire						
Self-injurious behavior	N (%)	11 (73.30)	4 (57.10)	*	*	.63
Physical aggression		6 (40.00)	2 (28.60)	*	*	1.00
Destruction of property		9 (60.00)	3 (42.90)	*	*	.65
Stereotyped behavior		9 (64.30)	3 (42.90)	*	*	.40
The Activity Questionnaire						
Impulsivity	Mean (SD)	14.17 (8.53)	12.57 (7.72)	.05	1,22	.82
Overactivity		15.87 (9.31)	7.86 (8.90)	.06	1,22	.35
Total score (excl impulsive speech)		30.04 (15.77)	20.43 (16.07)	.00	1,22	1.00
Mood, Interest and Pleasure Questionnaire –Short form						
Mood	Mean (SD)	19.29 (1.86)	21.17 (2.44)	1.96	1,21	.18
Interest & Pleasure		16.06 (4.47)	15.14 (4.63)	1.37	1,21	.26
Total score		35.34 (5.19)	36.31 (6.49)	.16	1,21	.70

Repetitive Behaviour Questionnaire						
Stereotyped behavior	Mean (SD)	6.93 (4.27)	4.50 (3.82)	.38	1,21	.55
Compulsive behavior		6.38 (8.69)	5.71 (4.38)	3.49	1,21	.08
Insistence on sameness		1.43 (1.99)	2.71 (2.89)	2.28	1,21	.15
Total score (excl verbal subscales)		16.57 (11.82)	15.29 (9.75)	2.26	1,21	.15

* Fishers exact; ** cut off scores reflect author suggested cut offs for further investigating the presence of ASD/autism. They do not reflect clinical diagnosis of ASD.
NIPBL-positive: participants with a confirmed *NIPBL* mutation (n=14) or classified by Average Face Analysis as NIPBL-like (n=1)
NIPBL-negative: participants who did not have a *NIPBL* mutation (includes those with *SMC1A* (n=3), *HDAC8* (n=1) mutations and those negative for all known CdLS mutations (n=3)

Table IV: Pearson correlations between chronological age and scores on behavior measures.

	NIPBL positive (n=15)	**NIPBL negative (n=7)**
Social Communication Questionnaire		
Social interaction	.07	-.80
Communication	.27	.11
Repetitive behavior	.15	.20
Total score	.31	-.26
The Activity Questionnaire		
Impulsivity	.03	.07
Overactivity	-.19	-.19
Total score (excl impulsive speech)	-.10	-.07
Mood, Interest and Pleasure Questionnaire –Short form		
Mood	-.18	-.06
Interest and Pleasure	**-.60***	.62
Total score	**-.58***	.42
Repetitive Behaviour Questionnaire		
Stereotyped behavior	.20	.45
Compulsive behavior	.25	.11
Insistence on sameness	**.35****	-.10
Total score (excl verbal subscales)	.31	.14

*** p <.05**
**** p ≤.01**
NIPBL-positive: participants with a confirmed *NIPBL* mutation (n=14) or classified by Average Face Analysis as *NIPBL*-like (n=1)
NIPBL-negative: participants who did not have a *NIPBL* mutation (includes those with *SMC1A* (n=3), *HDAC8* (n=1) mutations and those negative for all known CdLS mutations (n=3)